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Department of Biochemistry, Bharathidasan University Constituent College for Women, Orathanadu - 614 625, Tamil Nadu, India

Research Article

Phytochemical and Acetyl cholinesterase activity in leaf extract of *Ginkgo biloba* L.

S.Alagendran[1*], N.Pushpa[2]

[1] Crop Improvement Division, Adhiyamaan College of Agriculture and Research, Athimugam, Shoolagiri TK, Hosur, Krishnagiri– 635105, Tamil Nadu, India

[2] PG & Research Department of Microbiology, Cauvery College for Women, Annamalainagar, Trichy-620018, Tamil Nadu, India

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia in elderly humans. The plants have been chosen based on their use as memory enhancing as well as their nutrient value. These plants have been consumed as nutritious food and are believed to play an important role in health-promotion. The objectives of this study were to evaluate the phytochemical profiles in leaf extract of *Gingko biloba* and acetyl cholinesterase inhibitor in serum of 120mg/g treated with cognitive impairment of AD patients. Mini mental state examination (MMSE) shows the lower significant range in AD patients at P≤0.01. Phytochemical test was observed in the methanol extract, which contained a progression of flavonoids and phenolics in triplicates assay by TLC. The results were expressed as IC_{50} and the percent of inhibition of acetylcholinesterase (AchE) activity.

Keywords: *G. biloba* leaf extract, serum, acetyl cholinesterase, flavonoids

Introduction

Alzheimer's disease is a progressive, severe neurological chaos to occur little by little also outcome in memory loss, anomalous behavior and loss of the competence to belief [1-3]. Medicinal plants might be used in the treatment of learning and memory impairments. *Gingko biloba* extract considerably decline the AChE activity in the brain. The inhibition of AChE activity can be associated with development perceived in β-amyloid induced deficits in passive avoidance by *G. biloba* extract [4]. The degenerations in AChE activity show a proliferation in the basal level of acetylcholine. Brain areas associated with cognitive functions, particularly the neocortex and hippocampus, are the regions that mostly affected by the pathology which is characteristic of AD[2]. The main cure for AD is pharmacological treatment. Better understanding of the disease process and designed clinical trial are step forward and have improved related treatments for cognitive and noncognitive symptoms [5]. Pharmacological treatment strategies in AD include three set of treatment: 1) their mechanism is based on disease-modifying therapies such as vitamin E; 2) their mechanism is based on compensation of neurotransmitter such as a cholinesterase inhibitor; 3) psychotherapy factors that are prescribed for symptoms of conduct disorder[6]. AChE is predominantly originated on neuromuscular junctions and cholinergic synapses in the central nervous

system. Here, AChE hydrolyzes acetylcholine into choline and acetate after activation of acetylcholine receptors at the postsynaptic membrane. AChE activity serves to terminate synaptic transmission, preventing continuous nerve firings at nerve endings[7]. Further, the present, study proven the AD treatment strategy is cholinesterase inhibitors that can inactivate the acetylcholinesterase (AChE) enzyme in order to increase acetylcholine levels in the brain. Further, we examined the protective effects of *G. biloba* leaf extract through phytochemical assay and neurochemical approaches that retain dopaminergic characteristics and have been widely used for neuro protection.

Materials and methods

Ginkgo biloba belongs to the family is Ginkgoaceae. It has been used to treat neurological diseases such as learning deficits, loss of memory (dementia) and neurosensory disorders.

Plant Extraction of *Ginkgo biloba* leaf extracts

Dried 4 g of powdered plant material (leaves) was extracted by sequential cold maceration using dichloromethane, ethyl acetate, methanol and water blended with five volumes of all extracts (100 L) by shaking for 24 h at room temperature.120mg/g of leaves extract of *Ginkgo biloba* enhances with AD patients in three consecutive periods. Mini Mental State Examination(MMSE) examination also determined to find out the status of cognitive impairment and memory deficits (Score Shows efficacy in severe disease (MMSE.5-14).

Qualitative analysis of the MMSE[8].

The MMSE score has been analyzed both as a total score (range: 0-30) and a disaggregated score, as follows: temporal orientation (range: 0-5), spatial orientation (range: 0-5), registration (range: 0-3), attention (range: 0-5), delayed recall (range: 0-3), denomination (range: 0-2), sentence repetition (range: 0-1), obeying oral command (range: 0-3), reading and obeying command (range: 0-1), writing a sentence (range: 0-1), and copying a design (range: 0-1).

Phytochemical Analysis

All extracts were subjected to preliminary phytochemical screening for the determination of major secondary metabolites through TLC[9] .

Determination of Serum Acetyl cholinesterase inhibition (AChE) Activity

Serum AChE inhibition activity was measured using modified Ellman's colorimetric method. The acetylcholinesterase activity was measured by adding an artificial substrate analog of acetylcholine, acetylthiocholine (ATC) for every two min. Thiocholine released because of the cleavage of ATC by AChE is allowed to react with the –SH group of the reagent 5, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow colored anion with an absorbance maxima at 412 nm [10]. The acetyl cholinesterase inhibitory activity of the plant extracts was evaluated as the method previously described[11].

Results

Phytochemical analysis was represented in Table 1.Flavonoids imply the multipotent agents in combating Alzheimer's disease (AD) by enhancing acetylcholine levels. Among the 5 different concentrations, flavonoids were found to inhibit AChE with IC_{50} values ranging from 88 to 252.1 μg/mL in the inhibitory assay. The *Ginkgo biloba* leaf extract treat with AD patients was selected for IC_{50} Serum AChE inhibitory activity in consecutive of two fold concentrations of 32.3 μg/mL**.** The IC_{50} of the tested extracts and galantamine have specified in Table 2. The *Ginkgo biloba drug* exhibited the paramount inhibition against AChE with IC_{50} value of 4.68 μg/mL. The most inhibition of this extract was found to be 96.4% in the concentration of 40 mg/mL in serum samples of AD patients**.** The galantamine inhibited AChE both in serum and leaf extract of gingko biloba with a lower activity. The AChE inhibitory activity of the evaluated leaf methanolic extract of Ginkgo biloba at different concentrations (31.25, 62.5, 125, 250, and 500 μg/mL) showed that the extract has moderate inhibitory activity with the IC_{50} is 252.1 μg/mL (Table.3)**.**

Gingko biloba.L at different concentrations was examined in a set of experiments repeated three times; IC_{50} values of compounds represent the concentration of 50% detection limits.

Discussion

Analysis of MMSE to evaluate the pharmacological response, as well being a new approach, could give positive information both to better understand the

Table.1 Phytochemical analysis of *Ginkgo biloba* leaves part is used for invitro study

Phytochemicals	Methanol	Ethyl acetate	Dichloromethane	Yield (%)
Phenolics	++	++	+	9.56
Flavanoids	+++	+++	-	20.56
Saponins	+	++	+	4.35
Alkaloids	+ +	+	+	7.56
Terpenes	-	+	+	1.56

+++ *Presence* - *Absence*

Table.2 Acetyl cholinesterase inhibitory activities of methanolic extracts of *gingko biloba*

Sample	AChEI activity IC_{50} (µg/ml)	Galantamine
MMSE (Score range 5-14)	4.5	7.9
Serum	32.3±5.45*	2.58±0.76
Leaf extract of *gingko biloba*	4.68±1.05	1.02±0.03

** Values are mean ± SE of three times tests.*

Table.3 Extraction of *Gingko biloba*.L at different concentrations

Concentration (µg/mL)	Leaf extract of *gingko biloba* - AChEI activity IC_{50} (µg/ml)
31.25	88.5
62.5	252.1
125	101.4
250	112.6
500	133.1

characteristics of subjects with different responses and to quantify the AChEl effect on the cognitive performance. Our findings suggest that the improvement stimulate by the drug on the MMSE can be seen, in a short run, on selected item such as memory and orientation; these items are also the first to be exaggerated when looking at the natural history of the disease. On the long run, most of the item emphasized to be distorted and only a long-term follow up might give some indication on the constancy of these increased conditions. The items make use of the MMSE (delayed recall, attention, copying a design) as indicative for the performance in the selected cognitive domains because of the poor divergent validity of these items with the non-corresponding neuropsychological tests[12].

The phytochemical analysis evaluated by TLC showed the presence of flavonoids, phenolic acids, alkaloids and saponins in different extracts are determined in **Table 1.** Alkaloids were only present in methanolic extracts of all plant organs, while saponins only in aqueous extracts. Flavonoids were detected principally in ethyl acetate and methanolic extracts of leaves (yield contains 20.56%), and phenolic acids in ethyl acetate, methanolic extracts of all plant leaflets. The mean vs. standard error mean of AChE inhibitor possesses the IC_{50} 32.3 µg/ml in serum extracts of leaves showed the uppermost in methanol extracts of leaves in *Ginkgo biloba* while compare to other solvents recognized in **Table.2.** High AChE activity of these methanolic and ethyl acetate extracts is owed to the presence of flavonoids and phenolic acid. Mostly in G. biloba plant, several bioactive compounds were identified and quantified, such as flavonoids and phenolic compounds has potent antioxidants with an active role in relation to reducing the risk of atherosclerosis and attenuating neurological damage in patients with Alzheimer's disease.The linearity of scatter value showed AChE inhibitor activity is $Y=4.88x+17.51$; $R^2= 0.985$ of range of coefficient value is formed in methanolic extracts of leaves present in *Ginkgo biloba* in compared with serum aliquots considerably decline. The AChE inhibitory activity of test compounds revealed that flavonoids were found to inhibit AChE with IC_{50} values ranging from 57.8 to 133.1 µg/mL, Thus, a variety of mechanisms of neuronal degeneration in Alzheimer diseases has been suggested, including improvement of free radicals, oxidative stress, mitochondrial dysfunction, inflammatory processes, genetic factors, environmental impact factors, apoptosis, among oth-

ers[13]. Earlier studies conveyed that neuronal earliest changes and pathological alterations of this disease are interrelated to oxidative damage (with a very high input of oxidative stress), mainly in the development of neurogenetic abnormalities. Previous reports possess the results of AChEI, antioxidant and cytotoxicity activity of tested plants, can conclude that the ethanol extract of G. max which has been used widely as a dietary plant, was able to inhibit AChE significantly in vitro. Furthermore, this plant exhibited high antioxidant activity. This plant also showed no significant toxicity in the concentrations lower than 500-1000 µg/mL. Finally, the results of our work reveal that *G. biloba* would be a valuable source of anticholinesterase agents, with a potential use in pharmaceutical preparations. Noteworthy, secondary metabolites flavonoids, poly phenols, antioixidants containing rich food can also suggest that the soya-rich diets have health-promoting effects especially in elder people which are more prone to cognitive disorders.

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